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Molecular Heterogeneity in Mucoepidermoid Carcinoma: Conceptual and Practical Implications

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Abstract Mucoepidermoid carcinoma (MEC), the most common salivary gland malignancy of the upper aerodigestive tract and tracheobronchial tree, is also known for its considerable cellular heterogeneity including epidermoid, intermediate and mucin producing cells. Despite this structural and cellular heterogeneity, MEC is uniquely characterized by a specific translocation t(11; 19) (q12; p13), resulting in a fusion between the MECT1 and the MAML2 genes. Although the incidence of this fusion in MEC varies, it is generally accepted that more than 50 % of this entity manifest the *MECT1-MAML2*. Fusion-positive cases showed significantly better survival than fusion-negative cases, suggesting that MECT1-MAML2 represents a specific prognostic molecular marker in MEC. We contend that fusion in MEC represents a distinct mechanism in the development of this entity. In that context, fusion positive MEC, regardless of grade, manifest a more stable genome and better clinical behaviour, while fusion negative MEC represent a distinctly different pathway characterized by marked genomic instability and relatively aggressive tumors.

Keywords Mucoepidermoid carcinoma · t(11; 19) (q12; p13) · Fusion transcript · *MECT1-MAML2*

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Introduction: Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy of the upper aerodigestive tract and tracheobronchial tree. MEC is also known for its considerable cellular heterogeneity including epidermoid, intermediate and mucin producing cells. Conventional clinicopathologic parameters (patient age and disease stage and grade) have been reported to influence the biological behaviour and patient management. MECs are graded based on a three tiered system into low, intermediate and high grade, based on adverse features including: perineural invasion, angiolymphatic invasion, coagulative necrosis, high mitotic rate, cystic component <20 %, anaplasia, and infiltrative growth pattern. Considerable intra- and inter-observer variability are reflected by the reproducibility of histologic grading schemes [1, 2]. Interestingly, and despite these structural and cellular heterogeneity, MEC is uniquely characterized by a specific translocation, t(11; 19) (q12; p13).

Tumor-Specific Translocations and Fusion Oncogenes in Salivary Carcinomas

Chromosomal translocations are an uncommon feature of solid human tumors. Specific non-random chromosomal translocations with and without novel chimeric fusion oncogenes are unique genetic events of diagnostic, biological and therapeutic implications. The majority of these chromosomal rearrangements often lead to either the formation of potent fusion transcripts or the deregulation of tumor inducing genes. Fusion oncogenes are often derived from, and encode for, transcription factors, transcription regulators, and receptor tyrosine kinases, which are

frequently involved in oncogenesis. Currently, almost 400 critical gene fusions have been identified in human cancers; these oncogenes account for 20 % of human cancers [3], including salivary gland carcinomas-mucoepidermoid carcinoma (MEC) [4], adenoid cystic carcinoma (ACC) [5], hyalinizing clear cell carcinoma [6], and mammary analogue secretory carcinoma [7] (Table 1). Several fusion oncogenes have been identified in prostate cancers (TMPRSS2-ERG), non-small cell lung cancers (EML4-ALK, SLC34A2-ROS, or CD74-ROS), and in thyroid, renal, breast, salivary, and bronchial tissues [3, 8, 9].

t(11; 19) *MECT-MAML* Fusion Transcript in MEC

Mucoepidermoid carcinoma is characterized by the translocation of chromosomes 11q and 19p resulting in a fusion between the MECT1 and the MAML2 genes.

First described by Nordkvist et al. [4], the exact translocation was reported as the sole molecular event in all metaphases of a MEC and was suggested to be a critical event in the development of this entity [10]. Subsequently Tonon et al. [11] characterized the MECT1 and MAML2 as the underlying pathogenetic event in the majority of MECs. MECT1 (mucoepidermoid carcinoma translocated-1, also known as CRTC1, TORC1, and WAMTP1) is a 75-kD protein that activates c-AMP response element-binding (CREB)-mediated transcription [12]. MAML2 (mastermind-like 2) is a 125-kD protein involved in Notch signaling pathways. The *MECT1-MAML2* fusion protein is comprised of the N-terminal CREB protein-binding domain (exon 1) of MECT1 at 19p13 and the C-terminal transcriptional activation domain (exons 2–5) of the Notch coactivator MAML2 at 11q21 [11]. The *MECT1-MAML2* fusion protein may activate both cAMP-CERB targets and Notch signaling targets, with the resultant disruption of both cell cycle and differentiation functions [11]. Since CREB regulates cell proliferation and differentiation, and MECT1 deletion abolishes transforming activity, it is likely that CREB dysregulation mediates tumorigenesis. Moreover, Notch target genes, such as HES1 and HES5, are upregulated in fusion-positive MEC cell lines [11].

Evidence for the etiologic role of *MECT1-MAML2* in MEC include the following: (1) MECT1-MAML2 transforms rat RK3E cells in vitro and in vivo [13], (2) the fusion transcript has been identified in MEC-like tumors of the salivary glands, bronchopulmonary tree, thyroid, breast, skin, and cervix [11], and (3) RNA interference of the fusion transcript in t(11; 19)-positive tumor cells resulted in tumor growth suppression [14]. MECT1 protein can be detected in submandibular salivary gland epithelia during the early stages of morphogenesis but it disappears with acinar maturation [15]. Interestingly, the protein can be detected in tumors suggesting reactivation of dormant developmental gene. Although the incidence of this fusion in MEC varies, it is generally accepted that more than 50 % of this entity manifest the *MECT1-MAML2* [13, 16, 17]. Fusion-positive cases showed significantly better

Table 1 Tumor-specific fusion oncogenes in salivary carcinomas

	Mucoepidermoid carcinoma (MEC)	Adenoid cystic carcinoma (AdCC)	Hyalinizing clear-cell carcinoma (HCCC)	Mammary analogue secretory carcinoma (MASC)
Cell of origin	Precursor cells of exocrine glands in head and neck	Epithelial and myoepithelial cells of salivary glands	Epithelial (squamous) cells of salivary glands	Epithelial cells of salivary glands
Site of tumor	Exocrine glands in the upper aerodigestive tract and tracheabronchial tree	Salivary glands	Salivary glands oral cavity	Salivary glands
Pathognomonic translocation	t(11; 19) (q21; p13)	t(6; 9) (q22–23; p23–24)	t(12; 22) (q13; q12)	t(12; 15) (p13; q25)
Proto-oncogene	Mucoepidermoid carcinoma translocated-1 (MECT1)—also known as CRTC1, TORC1, WAMTP1	MYB	EWSR	ETV6
Promoter gene	MAML2	NFIB	ATF1	NTRK3
Fusion oncogene	MECT1-MAML2	MYB-NFIB	EWSR-ATF1	ETV6-NTRK3
Diagnostic modality	RT-PCR, FISH	RT-PCT, FISH, immunohistochemistry	RT-PCT, FISH	RT-PCT, FISH
Prognosis	t(11; 19) associated with improved survival	Histologic grade influences prognosis	Low-grade salivary carcinoma	Intermediate-grade salivary carcinoma (resembles secretory carcinoma of breast)

survival than fusion-negative cases, suggesting that MECT1-MAML2 represents a specific prognostic molecular marker in MEC. Behboudi et al. [16] reported median survival times of 10 and 1.6 years in fusion-positive and fusion-negative patients, respectively, as well as a significantly lower risk of local recurrence, metastases, or tumor-related death in fusion-positive patients, compared with fusion-negative patients. Recently, however, Seethala et al. [2] found that while translocation-positive patients had better disease-specific survival, disease-free survival was not significantly affected.

Other Genetic Events Associated with MEC

Anzick et al. [13] have identified deletions within the *CDKN2A/p16* gene in fusion-positive MEC cases with poor prognosis; CDKN2A deletion or hypermethylation was not detected in any fusion-positive MEC case with good prognosis. Fusion-negative cases with poor prognosis often showed CDKN2A deletion or methylation, suggesting that such CDKN2A alterations represent additional events in MEC tumorigenesis. It is likely that both *MECT1-MAML2* and CDKN2A status define more homologous prognostic categories of patients with MEC—a finding that may have biologic and therapeutic implications [13].

Other related but infrequently detected MEC-related fusions have recently been reported. Fehr et al. [18] have described a novel fusion between MECT3 and MAML2. The CRTC family includes 3 human genes: CRTC1 (a.k.a. MECT1), CRTC2 at 1q21, and CRTC3 at 15q26; MECT1 has 32 % homology with the latter two genes [12]. In a study of 101 MECs, Nakayama et al. [19] detected *MECT1-MAML2* and *CRTC3-MAML2* fusion transcripts in 34 and 6 % of cases, respectively. The two fusions were mutually exclusive, and *CRTC2-MAML2* fusions were not found in any MEC cases. There was no significant survival difference between patients with either translocation, but fusion-positive patients had better disease-free survival than fusion-negative patients [19].

Genomic Profiles and *MECT1-MAML2* Fusion Distinguish Different Subtypes of MEC

A recent genome wide comprehensive analysis of copy number alterations of MEC has reported that low-grade tumors were fusion positive whereas only 3 of 13 high-grade tumors were fusion positive [20]. The analysis revealed that fusion-positive tumors had significantly fewer copy number alterations (CNAs) compared with fusion-negative tumors (1.5 vs. 9.5; p 0.002). The most frequent CNAs detected were losses at 18q12.2-qter (including tumor suppressor genes *DCC*, *SMAD4* and *GALR1*), 9p21.3 (including tumor suppressor genes *CDKN2A/B*), 6q22.1–q23.1, and 8pter–p12.1, and gains of 8q24.3 (including oncogene MAFA), 11q12.3–q13.2, 3q26.1–q28, 19p13.2–p13.11, and 8q11.1–q12.2 (including the oncogenes *LYN*, *MOS*, and *PLAG1*). Based on these results it was proposed that MEC may be subdivided in (1) low-grade, fusion-positive MEC with no or few genomic imbalances and favorable prognosis, (2) high-grade, fusion-positive MEC with multiple genomic imbalances and unfavorable prognosis, and (3) a heterogeneous group of high-grade, fusion-negative adenocarcinomas with multiple genomic imbalances and unfavorable outcomes [20]. This study opens a new avenue, indicating that molecular genetic analysis can be useful adjunct to histologic scoring of MEC and may lead to development of new clinical guidelines for the management of these patients [20].

Although, these steps are in the right directions, we contend that fusion in MEC represents a distinct mechanism in the development of this entity. In that context, fusion positive MEC, regardless of grade, manifest a more stable genome and better clinical behaviour, while fusion negative MEC represent a distinctly different pathway characterized by marked genomic instability and relatively aggressive tumors. This contention is supported by our recent findings and those of others [20], where fusion positive tumors showed considerably lower copy number alterations than fusion negative MECs.

Clinical and Diagnostic Significance of Fusion Transcript *MECT-MAML*

The detection of this fusion transcript in a significant number of MECs may have an impact on diagnosis, prognosis, and treatment.

Fine-needle aspiration cytology (FNAC) is increasingly being used as part of a diagnostic triage for putative salivary gland tumors, although its true diagnostic role remains controversial. With respect to MEC, studies suggest that FNAC is considered diagnostically accurate in high- or intermediate-grade tumors but unsatisfactory for low-grade MEC. The application of molecular techniques to cytological material to detect the *MECT1-MAML2* fusion transcript/protein may be helpful in cases of uncertainty, although clinical studies are required to validate such an approach. The finding that high-grade MEC may also express the fusion transcript suggests that detection of the transcript may be helpful in distinguishing this tumor type from poorly differentiated adenocarcinoma or clear cell carcinomas when conventional histological distinction is difficult. High grade MEC is prone to diagnostic confusion with adenosquamous carcinoma, adenoid (acantholytic)

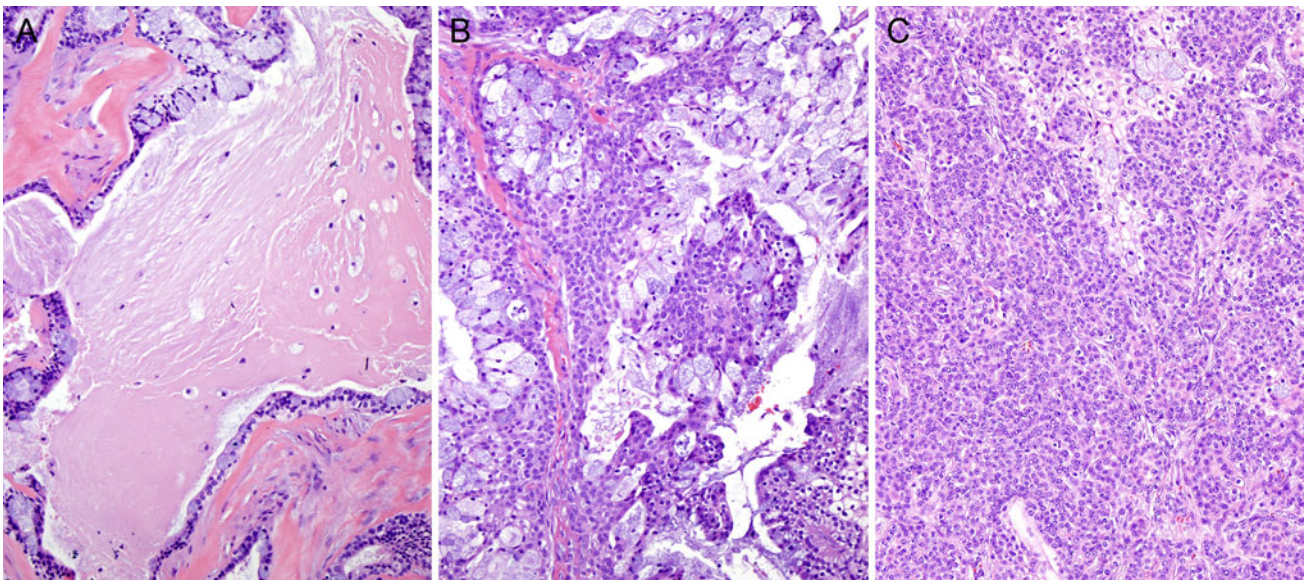

Fig. 1 Although in the initial series the fusion transcript was restricted to low (**a**) and intermediate grade (**b**) MEC, the translocation is also detected in high grade MEC (**c**)

squamous carcinoma, and less likely with adenocarcinoma NOS and salivary duct carcinoma [21]. Although in the initial series the fusion transcript was restricted to low and intermediate grade MEC, in our experience, and recently confirmed by others the translocation was also detected in high grade MEC (Fig. 1). The *MECT1-MAML2* fusion transcript is present in high grade MEC at a lower rate compared to low-intermediate grade MEC. While age, stage and grade are proven MEC prognosticators, even histologically low grade MEC may behave aggressively [21]. The most challenging category of MEC in terms of prognostic and management is the intermediate grade. Since histologic grading criteria are most useful tool in morphologic classification, the inclusion of molecular findings provide submicroscopic information for their better assessment. We argue that grading represents a continuum rather stage limited event, in the majority of MEC, and that the integration of the molecular finding allows for the biological stratification within individual grades. The translocation can be regarded as a biomarker of favorable prognosis, and this result may potentially influence the decision for performing a neck dissection or recommending radiotherapy. Despite retrospective data supports its value, prospectively the prognostic value of the translocation remains to be verified.

The presence of *MECT1-MAML2* in a significant proportion of MECs offers an avenue for an alternative treatment approach based upon its putative role in tumor initiation and progression. However, in MEC, the *MECT1-MAML2* fusion product is not itself an enzyme. Instead, it acts in conjunction with other proteins to form transcription activation complexes that act on Notch- and CREB-regulated pathways. Therefore, therapeutic targeting is less clear. To date, most attention has been paid to the role of Notch inhibition via a number of mechanisms. "Anti-Notch" agents under investigation for clinical use include γ-secretase inhibitors, although the finding that *MECT1-MAML2*–mediated Notch-target activation occurs in the presence of γ-secretase inhibitors would suggest that these agents are unlikely to be effective in MEC. More promising, although clearly still investigational, is the potential role for RNA interference strategies in the abrogation of tumor progression.

Although largely speculative at this point, MEC harboring the *MECT1-MAML2* translocation may be a valid target for tyrosine kinase inhibitor therapy. Two pulmonary MEC cell lines caring the translocation (H292, H3118) were shown to be sensitive to gefitinib [22], additional to anecdotal reports of pulmonary MEC with clinical response to gefitinib [23, 24].

In conclusion, in clinical practice, *MECT-MAML* fusion transcript is an ancillary test to be performed on paraffin tissue (RT-PCR, FISH). The translocation status is confirmatory support for the diagnostic of MEC, with respect to high-grade tumors and variant morphologies.

Conflict of interest None.

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